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MINIREVIEW

Catabolite Gene Activator Protein Activation of *lac* Transcription

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CAP ACTIVATION OF *lac* TRANSCRIPTION

What is the mechanism by which genes are positively regulated? How can several unlinked genes encoding related functions be regulated by a common signal? These are two of the questions which can be addressed by studying the catabolite gene activator protein (CAP). CAP responds to differences in the availability and nature of carbon sources, via variations in the intracellular concentration of cyclic AMP (cAMP). CAP, when complexed with cAMP, is a sequence-specific DNA-binding protein which activates several gene systems and represses others. It has been most extensively studied for *Escherichia coli*, although closely related proteins exist in other gram-negative bacteria.

CAP is an important paradigm for understanding the positive control of gene expression because of the extensive genetic, biochemical, and structural studies which have provided significant insights into its mode of action. This last year has been especially important because of several recent accomplishments: the determination of the molecular structure of the CAP-DNA complex (21), the genetic identification of a solvent-exposed loop between two pleated-sheet structures as being critical for positive activation (1, 5, 6), and the suggestion that the carboxy-terminal portion of the RNA polymerase α subunit may provide a contact point for CAP (12).

This review concentrates on analyzing how CAP activates *lac* operon expression. Although it is likely that CAP's mechanism of action will be similar to that of some other positive activator proteins, still others are likely to function quite differently. Moreover, CAP itself may act differently in different cases. As will be discussed below, CAP binds at different distances relative to the transcription start point for different systems; does this require a unique mechanism for each physical arrangement? Moreover, in some cases CAP acts through an additional "intermediate" protein, introducing another partner into the equation.

THREE MODELS FOR CAP ACTIVATION

It has long been suspected that CAP activates transcription initiation through a protein-protein contact with RNA polymerase (9). Perhaps this contact stabilizes binding of RNA polymerase to DNA by providing a contact in addition to those provided by the promoter sequences, or perhaps CAP induces a favorable conformational change in RNA polymerase. As described below, current evidence strongly favors a protein-protein contact as playing a major role in CAP activation.

An alternative model envisions CAP acting through the DNA, generating a distortion which facilitates transcription initiation (3). In fact, CAP causes a severe bend in the DNA upon binding, and this could lead to contact of upstream DNA with RNA polymerase (21, 25).

Finally, CAP acts as a repressor in some systems (18, 26). Since the *lac* promoter region (and other regulatory regions such as *gal*) contains several promoterlike elements which overlap the promoter (Fig. 1), it was thought that CAP could activate transcription by limiting the access of nonproductive competitive promoterlike elements to RNA polymerase (16).

WHY DIRECT CAP-RNA POLYMERASE CONTACTS ARE PROBABLY IMPORTANT FOR *lac* ACTIVATION

Several lines of evidence indicate that direct CAP-RNA polymerase contacts play an important role in *lac* activation. CAP and RNA polymerase each exert a mutually cooperative effect on the *lac* DNA binding of the other. This cooperativity was demonstrated by the observation that RNA polymerase stabilizes the interaction of CAP with its binding site as determined by detailed footprinting analyses (13, 20, 23). In addition, fluorescence polarization experiments demonstrated that CAP and RNA polymerase can interact in solution (dissociation constant, ~1 μM) (19).

Recent genetic studies have added important new evidence supporting the protein-protein contact model and have provided tools with which to test critically the inferences from biochemical experiments. Three groups have isolated CAP mutants which are selectively unable to activate transcription; that is, the mutant CAPs are functional as repressors but defective in activation (1, 5, 6). Two of the studies utilized randomized mutagenesis protocols designed to identify any possible site uniquely involved in positive control (5, 6). All of the resulting mutants have residue changes within the same region, between amino acids 156 and 162. Alanine substitution mutagenesis suggests that the threonine at position 158 is particularly important (5). These mutant CAPs are interesting because those which have been tested have normal DNA binding and bending properties in vitro (1, 5) and normally repress RNA polymerase interaction with competing promoters in vivo (6). As shown in Fig. 2, these mutants define a surface-exposed loop which is located some 15 Å (1.5 nm) away from the DNA in the bound complex (21).

It is proposed that this peptide loop is the contact domain for RNA polymerase. An obvious test of this proposal is to ask how the mutants (which are unable to activate transcription) affect the in vitro interaction of CAP and RNA polymerase. Preliminary studies from Richard Ebright's laboratory indicate that mutant CAP is defective in interacting with RNA polymerase in vitro (5). This result strongly suggests that the model which we are developing based on *lac* is likely to be important for some other CAP-activated systems.

FIG. 1. *lac* promoter elements. The *lac* promoter is located within a complex arrangement of DNA sequences. The major promoter (P1) programs the synthesis of β-galactosidase as well as the permease and transacetylase. It is activated by CAP and repressed by the *lac* repressor. The P1 mRNA start site is at +1. The relevant −10 and −35 recognition sequences are indicated with open boxes. Overlapping P1 are two upstream promoterlike elements, P2 and P3. P2 and P3 program very low levels of *lac* mRNA in vivo (26). P2 is a major in vitro binding site for RNA polymerase in the absence of CAP (4, 6, 7, 16). CAP binding represses RNA polymerase interaction with P2 and P3 (6, 16, 26).

Finally, the direct protein-protein contact model has been supported by genetic and biochemical analyses of the other partner in the proposed interaction, RNA polymerase. Igarashi and Ishihama have reported in vitro experiments showing that holoenzyme containing carboxy-terminal deletions of the α subunit transcribes several CAP-independent promoters normally but fails to demonstrate CAP activation of *lac* (12). A point mutation analysis of the α subunit may indicate the precise contact point(s) involved in CAP's activation of *lac*. (It should be noted, however, that these α deletions did not perturb CAP's activation of *gal* P1 [11]!)

WHY DNA BENDING MIGHT CONTRIBUTE TO CAP ACTIVATION OF *lac*

CAP bends the DNA associated with its target DNA sequence by 90° or more (21, 25). It is very tempting to presume that this DNA bending plays a critical role in transcription activation. The role could be to position CAP in an optimal configuration in order to make the CAP-RNA polymerase specific interprotein contact. Alternatively, the DNA bend itself might play a role in transcription activation, perhaps by providing upstream DNA-RNA polymerase contacts or by relieving an energy barrier to transcription initiation resulting from the constraints imposed in vivo by DNA superhelicity (8).

The simplest conclusion from recent experiments regarding the *lac* operon is that the DNA bending activity and upstream DNA-RNA polymerase contacts, in particular, are not sufficient to activate transcription. As has been noted above, several CAP positive control mutants have been found to bind and bend DNA normally; therefore, bending of DNA by CAP does not suffice to activate *lac* transcription in vivo (1, 5). In addition, the possible requirement for upstream contacts has been directly tested in in vitro experi-

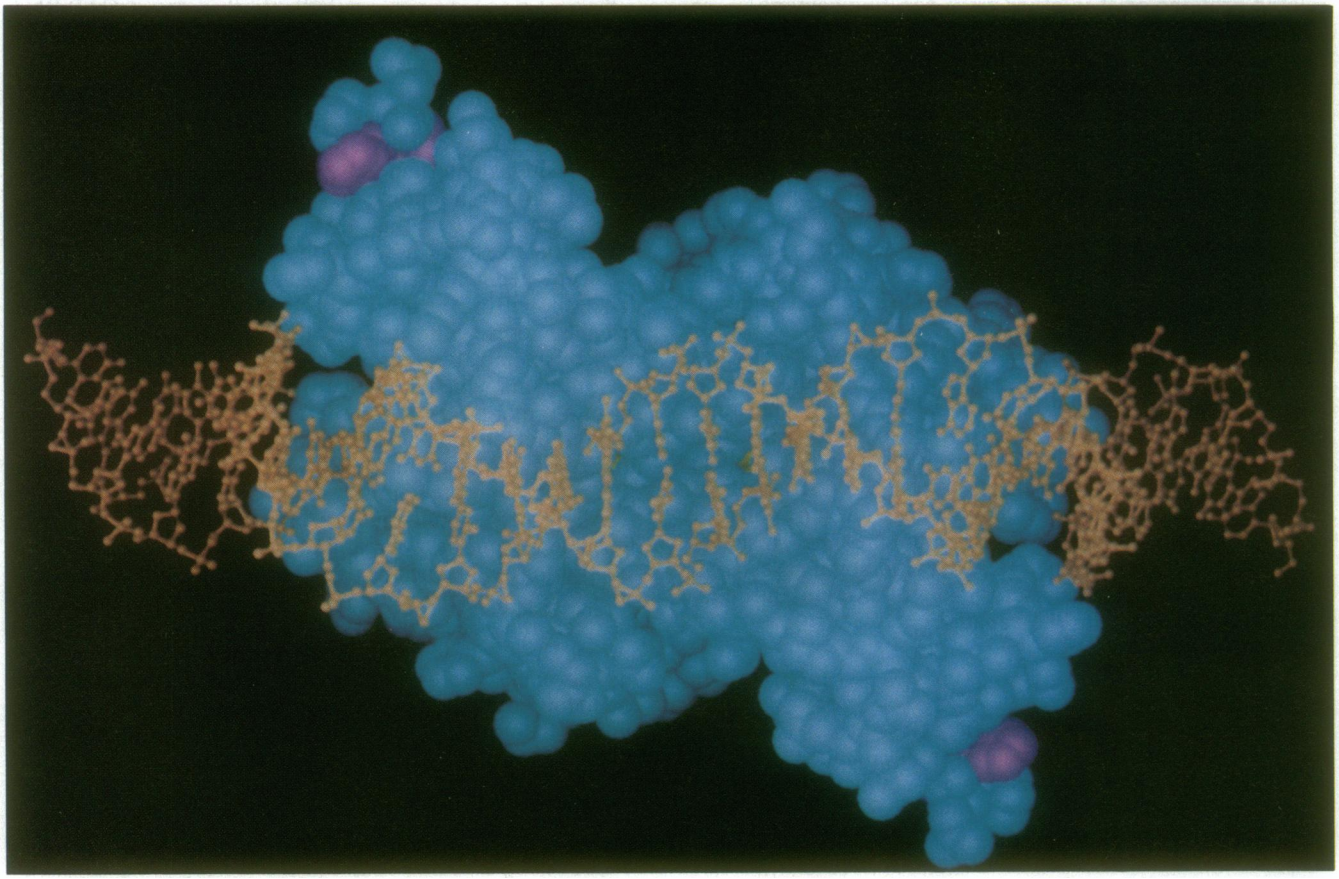

FIG. 2. CAP bound to its DNA recognition site. A space-filled model of CAP bound to its target DNA site is pictured, with the DNA in front of the CAP protein dimer. The DNA molecule is bent away from the viewer on either side of CAP. The His-159 and Gly-162 residues, sites of positive control mutations (1, 5, 6), are highlighted. These mutations and others from positions 156 to 162 may define a contact region for the α subunit of RNA polymerase. The figure was provided by T. Steitz and is derived from the structural analysis of the CAP-DNA complex by Schultz et al. (21). Note that the location of these residues, though not close to the DNA, is on the "DNA side" of CAP, implying that the RNA polymerase α subunit would reach underneath CAP.

ments by using defined *lac* promoter DNA fragments. Removal of DNA upstream of −83 has little or no effect on CAP's ability to stimulate RNA polymerase binding to or transcription from the *lac* promoter (7, 23).

A contributory role for DNA bending cannot be ruled out, however. In particular, a possible energetic role for DNA bending when the DNA is in a superhelical configuration has been proposed (8). Moreover, in another CAP-stimulated system (similar to *gal* in structure), curved upstream DNA sequences can enhance expression in vivo (but not in vitro) in the absence of CAP (2). This may reflect the differing architecture of the systems (the CAP DNA-binding site is centered at −41.5 as opposed to −61.5) or may be irrelevant to CAP's mode of action, suggesting rather an alternative mechanism of activating transcription.

WHY A REPRESSION CASCADE IS NOT LIKELY TO CONTRIBUTE SUBSTANTIALLY TO ACTIVATION

The observation that overlapping promoterlike elements (P2 and P3) exist upstream of the *lac* promoter (P1) (Fig. 1) and that CAP binding represses polymerase interaction with P2 and P3 suggested that CAP might act through a repression cascade; e.g., CAP would repress RNA polymerase binding to P2 and P3, which would relieve interference by RNA polymerase bound at P2 or P3 with RNA polymerase's binding to P1 (16). Current evidence suggests that this cascade, if it exists, is unlikely to play a substantial role in *lac* P1 activation by CAP. Mutations which inactivate P2 have no measurable effect on CAP activation of *lac* P1 expression (4). Moreover, the CAP mutants defective in positive control are fully functional in P2, P3 inactivation (6); thus, this repression alone is not sufficient to provide activation.

HOW CAN THE DIFFERING ARCHITECTURES OF CAP ACTIVATED SYSTEMS BE UNDERSTOOD?

This review has focused on the *lac* system, but of great interest is whether what we have learned for *lac* can be generalized to other systems. Of particular concern are the obvious differences in the controlling element architecture (e.g., −61.5 for *lac* [3], −41.5 for *gal* [24]) and the apparent differences in the step in transcription initiation activated in different systems (RNA polymerase binding, closed-complex isomerization, or escape from the initiation to the elongation complex [10, 14, 15, 17]). The evidence seems confusing. For instance, the architecture suggests significantly different potential overlaps between the two proteins. Moreover, studies by Igarashi and Ishihama indicated that the α carboxy-terminal deletions generate a holoenzyme which appears to be functional for the 41.5-base-spaced CAP activation but nonfunctional for the *lac* case (11, 12).

However, from the vantage point of CAP, we believe that the same contact is used in all systems. CAP positive control mutants are defective for all tested positive activation systems. Moreover, the observation that these mutants also block in vitro CAP-RNA polymerase interaction suggests that the 156-162 loop is the primary site for RNA polymerase contact.

These apparently conflicting observations might be resolved by one or both of the following explanations. (i) CAP enhances transcription initiation through one of several possible contacts with RNA polymerase, all using the same contact on CAP but different contacts on RNA polymerase. This possibility is clearly suggested by the recent α subunit deletion studies of Igarashi et al. (11). (ii) CAP enhances transcription initiation through a contact between the same protein domains in all cases. The differing promoter architectures appear to make this an unlikely model. However, we may find that the *gal* activation by a −41.5-centered CAP in fact requires a second CAP bound immediately upstream of the first and that this second CAP actually provides the contact point for RNA polymerase (see reference 22 for data regarding this possibility). Thus, a more detailed analysis of apparently differing systems may yet reveal very similar mechanisms.

Our uncertainties regarding CAP activation of its various target promoters are both a matter of confusion and a wonderful opportunity to do some exciting work. It is likely that other transcription activator proteins will also present a complicated picture; however, in this case the tools to resolve the questions are clearly at hand.

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